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The host-pathogen interaction in *Campylobacter jejuni* infection of chickens: an understudied aspect that is crucial for effective control

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Campylobacter jejuni, invasion, chicken, innate immunity, cytokines.

Campylobacter jejuni is the main causative agent of food-borne diarrhoeal illness in the UK and most of the developed world. In addition, it is a significant cause of morbidity and mortality in infants in the developing world. It causes symptoms that range from mild, self-limiting disease to more severe haemorrhagic diarrhoeal disease that can last up to two weeks and in some cases even relapse¹. In addition, *C. jejuni* can also cause long-lasting sequelae, which include Guillane-Barré syndrome and reactive arthritis². In the UK alone, the total burden of campylobacteriosis is estimated to reach over 700,000 cases per year³, at an estimated cost to the economy of £500 million⁴. The main source of campylobacteriosis is the consumption of contaminated poultry which has been attributed to up to 80% of human infections⁵. In the UK, a recent year-long survey estimated that, in 2014, an average of 70% of poultry carcasses on retail were contaminated with *Campylobacter*⁶. Even though it is the main zoonotic food-borne pathogen, the biology of *C. jejuni* and its interactions with the chicken's immune system is not as well understood as other pathogens, such as *E. coli* and *Salmonella*. In chickens, *Campylobacter* resides in the intestinal mucus and invasion levels are low¹. Historically, *C. jejuni* was described as a commensal organism in chickens, as it does not cause overt clinical signs. However, *C. jejuni* can be isolated from the liver of infected chickens, which is the main cause of campylobacteriosis outbreaks in the UK³; this internal organ infiltration is not typically considered commensal behaviour. The reasons behind *C. jejuni*'s biology of infection in chickens are still not well understood and the relative contributions of the chicken's immune response to infection vs. the ability of *Campylobacter* to invade and to evade or subvert the immune response are still not well characterised.

In this issue of Virulence, Vaezirad *et al*⁷ describe how treatment of chickens with glucocorticoids (GC), which induce generalised immunosuppression, influences the invasive behaviour of *C. jejuni*. The data suggest that *C. jejuni* has the intrinsic ability to translocate the intestinal barrier and disseminate to the liver. However, the chicken is able to mount an effective early innate immune response which appears to be partially responsible for limiting the amount of tissue and organ infiltration in chickens. This study demonstrated that, following GC treatment, both caecal colonisation levels and the invasion of *C. jejuni* into the liver were significantly increased when monitored daily during the first four days after infection. To my knowledge, this is the first publication to demonstrate an increased colonisation and invasion of *C. jejuni* within the liver following experimental immunosuppression in chickens.

To demonstrate the induction of an innate immune response following *C. jejuni* infection, Vaezirad and co-workers⁷ measured the induction of pro-inflammatory gene expression after infection. Following *C. jejuni* infection of non-GC treated chickens, statistically significant increases of various magnitude in the pro-inflammatory cytokines IL-8, IL-6, IL-1 β and iNOS were observed in both the spleen and the liver at days 1 and 4 after infection. No increase in IFN β was observed in these birds. GC treatment without *C. jejuni* infection was shown to induce significant decreases in the expression of these genes at various time-points in the caecum but no changes in expression of these genes were observed in the spleen. When the expression level of these genes was compared in GC-treated and non-treated chickens, both *C. jejuni* challenged, reductions in the expression of most of these gene were observed at both time-points and in both organs, suggesting effective suppression of the innate immune response mounted against *C. jejuni* infection. These results demonstrate that chickens are able to mount an effective early innate immune response against *C. jejuni* infection and that this response is ablated following immunosuppression with GC treatment. They also confirm that gene expression for the IL-8, IL-6 and IL-1 β cytokines, which were observed to be significantly induced in chicken cell lines *in vitro*⁸, is also induced *in vivo*. Gene expression during *Campylobacter* infection was compared to that induced by *Salmonella* Enteritidis and pathogen specific differences were observed.

The present study by Vaezirad *et al*⁷ provides another experimental confirmation that *C. jejuni* is not merely a commensal organism in chickens. Previously, Bull *et al*⁹ reported an association between *C. jejuni* positivity and an increase in the incidence of pododermatitis in commercial flocks. The causative link between *C. jejuni* infection and pododermatitis was later and for the first time proven experimentally by Humphrey *et al*¹⁰, who also revealed differences in the immune response to *Campylobacter* infection in different breeds of chickens. Humphrey *et al*¹⁰ highlighted that increased IL-10 gene expression at 12 days post infection (dpi) resulted in reduced intestinal pathology and lower incidence of pododermatitis. Further, it has been shown that chickens can produce high levels of IL-10 early in infection with other pathogens. For example, Setta *et al*¹¹ described a significant induction of IL-10 at 4 dpi with *S. Enteritidis* (which colonises the intestines but does not invade significantly or cause clinical signs) but not after infection with *S. Gallinarum* or *S. Pullorum* (which cause typhoidal-like disease but do not colonise the intestines). In contrast, Shaughnessy *et al*¹² showed that, even though both activate TLR-4 to similar levels, *S. Typhimurium* (which, like *S. Enteritidis*, colonises the intestines but does not cause clinical signs) but not *C. jejuni*

infection induces a significant increase in the expression of IFN γ . Increased IFN γ gene expression has been correlated with a decrease in severity of clinical signs due to campylobacteriosis in humans, supporting the hypothesis that a Th1-polarised immune response has the primary role in acquired immunity to *C. jejuni*¹³. Taken together, these observations suggest that a finely balance induction of both IL-10 and IFN γ may be required for the clearance of bacterial intestinal pathogens in chickens. Given this, an insight into the expression of this cytokine early in infection with *C. jejuni* would be valuable information, a cytokine that the authors unfortunately did not look at in the present study. As a result, further work investigating the expression of both IL-10 and IFN γ along the course of *C. jejuni* infection is required to assess the role of these cytokines in this infection.

While this study adds a valuable insight into the biology of *C. jejuni* infection in chickens, the fine mechanistic details of the interaction of *C. jejuni* with the chicken immune response remain to be elucidated. To gain some insight into the mechanisms behind their observation, Vaezirad *et al*⁷ investigated the induction of iNOS in two macrophage cells lines *in vivo* following GC-treatment and *C. jejuni* stimulation. They demonstrated that iNOS gene expression was abolished in GC pre-treated cells compared to non-treated control cells. This observation suggests a role for macrophages in the defence against *C. jejuni* invasion in chickens. However, *C. jejuni* survival ability within these GC-treated macrophage cell lines was not assessed. Furthermore, these observations were not validated *in vivo*, possibly due to the lack of widely available tools. The use of recently developed tools such transgenic chickens that express fluorophores on the cells of the macrophage lineage¹⁴ and a *C. jejuni* 11169H strain that expresses GFP stably and to high levels from a chromosomal integration¹⁵ could facilitate a more precise study of interactions between *C. jejuni* and macrophages and other cells of the immune system *in vivo*.

Overall, the nature of the protective immune response required to avoid *C. jejuni* colonisation remains to be fully elucidated. Previous studies looked at a limited number of genes over a limited time-course using different breeds of chickens. As such, further studies investigating the expression of an increased number of immune genes, both early and late in the course of the same infection, in a single breed of chickens, are required to characterise host-pathogen interactions in more detail. These should be complemented by *in vivo* studies of changes in populations of immune cells during *C. jejuni* infection in order to determine whether changes that may be observed at the level of

gene expression are due to true changes in gene expression or local changes in cell populations. Such information could aid the rational development of control strategies such as vaccination. Vaccines have been proven to be protective by independent research groups^{16,17,18}, however, the nature of the protective immune response remains to be elucidated. Recently developed transgenic chickens that lack functional antibodies¹⁹, would provide a valuable insight into the nature of protective immune response following vaccination.

In summary, while the data presented by Vaezirad *et al*⁷ represents a valuable addition to our understanding of the early host-pathogen interactions in *Campylobacter* infection in chickens, there are still major gaps in our understanding of the interaction of *C. jejuni* with the immune system of the chicken. Only with a more detailed study of these basic aspects we hope to rationally develop control strategies and even make effective vaccination feasible.

Disclosure of interest:

The author reports no conflict of interest.

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